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In re application of

DELFOURNE et al. Conf. 3819

Application No. 10/049,381 Group 1625

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PHENANTHROLINE-7-ONE DERIVATIVES AND THEIR THERAPEUTIC APPLICATIONS

DECLARATION UNDER RULE 132

Assistant Commissioner for Patents May 20, 2004
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Françoise COLLIGNON,

residing at: 11 Parc de Diane, 78350 Jouy-en Josas, FRANCE, having the degrees as a Pharmacist (Pharm.D.- Faculty of Pharmacy in PARIS) and as a Ph.D. in Chemistry and Biochemistry (Paris XI- ORSAY), working for CEPHALON France (formerly Laboratoire Louis LAFON), as Pharmacist in charge of Patents, declare:

I have a Ph.D. degree in Chemistry and Biochemistry and I graduated from the Faculty Pharmacy in Paris. I have been working in the Pharmaceutical Industry since 1983, successively as a Research Fellow, then as Research Fellow Senior, Section Manager and Project Team Leader. Recently, I took charge of the Patent Department of my Company. Through my career in the

Pharmaceutical Industry, I have reached a scientific expertise in various therapeutic areas notably in oncology being involved in the Research and Development programs of new chemical entities (NCEs) as anticancer drugs.

As a result of my education and professional experience, I consider myself capable of interpreting technical literature and understanding which the disclosed results can teach to those of ordinary skill in the art of antitumoral medicines.

I am aware of the fact that Bracher et al., *The structure of Neocalliactive Acetate - Proof by Total Synthesis (June 25, 1992)*, and *Schmitz, Francis J. et al., Biologically Active Compounds From Marine Organizers (1990), Pure and Applied Chemistry*, have been cited in the course of examination of this application and that it was alleged that the claimed composition was obvious in view of these references.

Experimental data have been submitted to me for my review and analysis. These results show that the activity of claimed compounds, notably CRL 8248, CRL 8325, CRL 8347, CRL 8406, CRL 8407, CRL 8416 and CRL 8422 is unexpectedly much more superior than the activity of ascididemin.

The compounds CRL 8248, CRL 8325, CRL 8347, CRL 8406, CRL 8407, CRL 8344, CRL 8422 and CRL 8534 were tested versus ascididemin as reference and versus a control group on various

models of tumors, namely lymphoma (L 1210 sc), carcinoma (MXT-HS) and (MXT HI) and melanoma (B16).

The hereafter obtained results show that the tested claimed compounds display an antitumoral activity within the experimental conditions of the assays. These compounds exhibit a decrease in tumor size whatever the model of tumor. On the opposite, this effect was not significant with ascididemin.

1) Lymphoma (L 1210 sc)

The compounds were tested on a model of lymphoma developed according to:

i) Malonne H, Farinelle S, Decaestecker C, Gordower L, Fontaine J, Chaminade F, Saucier JM, Atassi G and Kiss R

« In vitro and in vivo pharmacological characterization of the antitumour properties of two new olivacine derivatives, S16020-2 and S30972-1 ».

Clin Cancer Res 6:3774-3782, 2000

ii) Lesueur-Ginot L, Demarquay D, Kiss R, Kasprzyck PG, Dassonneville L, Bailly C, Camara J, Lavergne O and Bigg DCH

« Homocamptothecin, an E-ring modified camptothecin with enhanced lactone stability,

retains topoisomerase I-targeted activity and antitumour properties ».

Cancer Res 59:2939-2943, 1999

The tumor size parameter was selected as primary endpoint and studied at a DMT/4 dosage.

In contrast with the reference compound ascididemin that had no significant effect on tumor size in this model, CRL 8248 resulted in a significant decrease of tumor size by 25 % (Table 1).

Table 1

Treatments	L1210 sc model Decrease in Tumor size (%)
Ascididemin MTD/4 (3x3) 5 mg/kg starting on Day 7 post grafting	-3 (NS)
CRL8248 MTD/4 (3x3) 40mg/kg starting on Day 7 post grafting	-25 (p=0.0011)

NS : not significant.

- n= 9 B6D2F1 mice bearing tumor per treatment groups and control group
- Statistics: Mann-Whitney test.

In the experiments, the dose schedules were:

Control group treatment: 3 ip injections per week during 3 weeks (3x3) injections of physiological saline containing the solvent used to dissolve the compounds (starting on Day 7 post grafting).

Treatment groups

- Ascididemin MTD/4 (3x3) 5mg/kg = 3 ip injections per week during 3 weeks at a 5 mg/kg dose.

- CRL8248 MTD/4 (3x3) 40mg/kg = 3 ip injections per week during 3 weeks at a 40 mg/kg dose.

(injections starting on Day 7 post grafting).

2) Carcinoma (MXT -HS) and (MXT -HI)

The compounds were tested on a model of carcinoma sensitive (MXT-HS) and insensitive MXT-(HI) to hormones developed according to:

i) Watson CS, Medina D and Clark JH
"Estrogen characterization in a transplantable mouse mammary tumor"
Cancer Res 37:3344-3348, 1977

ii) Briand P
"Hormone-dependent mammary tumors in mice and rats as a model for human breast cancer (Review)"
Anticancer Res 3:273-282, 1983

iii) Danguy A, Kiss R, Leclercq G, Heuson JC and Pasteels JL
"Morphology of MXT mouse mammary tumors. Correlation with growth characteristics and hormone sensitivity"
Eur J Cancer Clin Oncol 22:69-76, 1986

i) Carcinoma (MXT-HS)

The tumor size parameter was selected as primary endpoint of the *in vivo* antitumor activity.

CRL 8325, CRL 8347, CRL 8406 and CRL 8407 induced a significant decrease of tumor size by 20 to 30% at an equivalent MTD/4 dose value in the implanted-tumor xenograft bearing mice model. In contrast, the reference compound ascididemin treatment had no significant effect on tumor size in this model.

Table 2

Treatments	MXT -HS model Decrease in Tumor size (%)
Ascididemin MTD/4 (3x3) 5 mg/kg starting on Day 7 post grafting	+1 (NS)
CRL8325 MTD/4 - (3x3) 40mg/kg starting on Day 7 post grafting	- 19 (p=0.02)
CRL8347 MTD/4 - (3x3) 40mg/kg starting on Day 7 post grafting	- 20 (p<0.05)
CRL8406: MTD/4 - (3x3) 40mg/kg starting on Day 7 post grafting	- 28 (p<0.05)
CRL8407: MTD/4 - (3x3) 40mg/kg starting on Day 7 post grafting	- 29 (p<0.02)

- NS : not significant.
- n= 9 B6D2F1 mice bearing tumor per treatment groups and control group
- Statistics: Mann-Whitney test.

In the experiments, the dose schedules were:

Control group treatment: 3 ip injections per week during 3 weeks (3x3) injections of physiological saline containing the solvent used to dissolve the compounds (starting on Day 7 post grafting).

Treatment groups

- Ascididemin MTD/4 (3x3)5 mg/kg = 3 ip injections per week during 3 weeks at a 5 mg/kg dose.

- CRL8325 MTD/4 (3x3) 40mg/kg, CRL8347 MTD/4 (3x3) 40mg/kg, CRL8406: MTD/4 (3x3) 40mg/kg and CRL8407: MTD/4 - (3x3) 40mg/kg = 3 ip injections per week during 3 weeks at a 40 mg/kg dose.

(injections starting on Day 7 post grafting).

ii) Carcinoma MXT -(HI)

The *in vivo* antitumor activity, using the tumor size parameter as primary endpoint, was characterized at the doses of MTD/4.

Results reported table 3 show that, CRL8344 decrease significantly the tumor size in the implanted-tumor xenograft bearing mice model by 15% as compared to the reference compound. Nevertheless, in this model, there is a trend to an increase of tumor size (although not significant) following treatment by the reference compound ascididemin data.

Table 3

Treatments	MXT-HI model Decrease in Tumor size (%)
Ascididemin (3x3) MTD/4 5 mg/kg starting on Day 7 post grafting	+23 (NS)
CRL8344 (3x3) MTD/4 40mg/kg starting on Day 7 post grafting	-15 (P=0.002)

- NS : not significant.
- n= 9 B6D2F1 mice bearing tumor per treatment groups and control group.
- Statistics: Mann-Whitney test

Treatment groups

- Ascididemin (3x3) MTD/45mg/kg = 3 ip injections per week during 3 weeks at a 5 mg/kg dose.

- CRL8344 (3x3) MTD/4 40mg/kg = 3 ip injections per week during 3 weeks at a 40 mg/kg dose.

(injections starting on Day 7 post grafting).

3) Melanoma (816) model

The compounds were tested on a model of melanoma developed according to:

i) Gibson MH, Bertalanffy FD.
« In vivo synchrony of solid B16 melanoma by cytosine arabinoside, an inhibitor of DNA synthesis »
J Natl Cancer Inst 1972,49(4): 1007-1018.

ii) Pasztor LM, Hu F.

« An amelanotic variant of B 16 malignant melanoma »

Cancer Res. 1972,32(8): 1769-1774.

iii) Hiscott J Duguay D, Mercier F, Stagg J, Martineau D, Bramson J, Servant M, Lin R, Galipeau J,.

« In vivo interferon regulatory factor 3 tumor suppressor activity in B 16 melanoma tumors »

Cancer Res. 2002, 62(18): 5148-5142.

The *in vivo* antitumor activity, using the tumor size parameter as primary endpoint, was characterized at the doses of MTD/4.

Results reported in table 4 show that, CRL8248, CRL8325, CRL8422 and CRL8534 decreased significantly the tumor size parameter by 32 to 56 % in the implanted-tumor xenograft bearing mice model. In contrast, the reference compound Ascididemin treatment was devoid of any significant effect on the tumor size parameter.

Table 4

Treatments	816 Decrease in Tumor size (%)
Ascididemin MTD/4 (3x3) 5 mg/kg starting on Day 7 post grafting	-13 (NS)
CRL8248 MTD/4 (3x3) 40mg/kg starting on Day 7 post grafting	- 56 (p=0.013)
CRL8325 MTD/4 (3x3) 40mg/kg starting on Day 7 post grafting	- 32 (p=0.04)
CRL8422 MTD/4 (3x3) 40mg/kg starting on Day 7 post grafting	- 40 (p=0.005)
CRL8534 MTD/4 (3x3) 40mg/kg starting on Day 7 post grafting	- 41 (p=0.02)

- NS : not significant.
- n= 9 B6D2F1 mice bearing tumor per treatment groups and control group.
- Statistics: Mann-Whitney test

In the experiments, the dose schedules were:

Control group treatment: 3 ip injections per week during 3 weeks (3x3) injections of physiological saline containing the solvent used to dissolve the compounds (starting on Day 7 post grafting).

Treatment groups

- Ascididemin MTD/4 (3x3)5 mg/kg = 3 ip injections per week during 3 weeks at a 5 mg/kg dose.

- CRL8248: MTD/4 (3x3) 40mg/kg, CRL8325: MTD/4 (3x3) 40mg/kg, CRL8422 MTD/4 (3x3) 40mg/kg, and CRL8534: MTD/4 (3x3)

40mg/kg = 3 ip injections per week during 3 weeks at a 40 mg/kg dose.

(injections starting on Day 7 post grafting).

As a conclusion, these *in vivo* results show that the claimed compounds display an unexpected antitumoral activity. They more particularly exhibit a significant decrease of the tumor size. Such antitumoral activity was not demonstrated for the reference compound (ascididemin) at DMT/x similar dose.

The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this day of June 3, 2004.

Françoise COLLIGNON